

CLAIMS

What is claimed is:

1. A method of screening for an agent that modulates the uptake of glutamate into a cell, said method comprising:
 - 5 i) contacting a cell comprising a nucleic acid selected from the group consisting of VGLUT1, VGLUT2, and VGLUT3 with a test agent; and
 - ii) detecting expression or activity of VGLUT1, VGLUT2, or VGLUT3 where an increase or decrease in the expression or activity of VGLUT1, VGLUT2, or VGLUT3 as compared to a control indicates that said test agent modulates the uptake of glutamate into a synaptic vesicle.
- 10 2. The method of claim 1, wherein said control is a negative control comprising contacting a cell at a lower concentration of said test agent.
3. The method of claim 2, wherein said lower concentration is the absence of said test agent.
- 15 4. The method of claim 1, wherein said cell is a somatic cell.
5. The method of claim 1, wherein said cell is an oocyte.
6. The method of claim 1, wherein said cell is a nerve cell.
7. The method of claim 1, wherein said cell is a vertebrate cell.
8. The method of claim 7, wherein said cell is a mammalian cell.
- 20 9. The method of claim 7, wherein said cell is a human cell.
10. The method of claim 1, wherein said detecting comprises detecting a VGLUT1 nucleic acid, a VGLUT2 nucleic acid, or a VGLUT3 nucleic acid.
11. The method of claim 1, wherein said detecting comprises detecting a VGLUT1 polypeptide, a VGLUT2 polypeptide, or a VGLUT3 polypeptide.

12. The method of claim 1, wherein said detecting comprises measuring activity of a VGLUT1 polypeptide, a VGLUT2 polypeptide, or a VGLUT3 polypeptide.

13. The method of claim 10, wherein said detecting a VGLUT1 nucleic acid, a VGLUT2 nucleic acid or a VGLUT3 nucleic acid comprises a nucleic acid 5 hybridization.

14. The method of claim 10, wherein said detecting a VGLUT1 nucleic acid, a VGLUT2 nucleic acid or a VGLUT3 nucleic acid comprises a method selected from the group consisting of a Northern blot, a Southern blot using DNA derived from the VGLUT1, VGLUT2, or VGLUT3 mRNA, an array hybridization, an affinity 10 chromatography, and an in situ hybridization.

15. The method of claim 10, wherein said detecting a VGLUT1 nucleic acid, a VGLUT2 nucleic acid or a VGLUT3 nucleic acid comprises a nucleic acid amplification.

16. The method of claim 11, wherein said detecting a VGLUT1 polypeptide, a VGLUT2 polypeptide, or a VGLUT3 polypeptide comprises a method 15 selected from the group consisting of capillary electrophoresis, Western blot, mass spectroscopy, ELISA, immunochromatography, thin layer chromatography, and immunohistochemistry.

17. The method of claim 12, wherein said measuring activity of a VGLUT1 polypeptide, a VGLUT2 polypeptide or a VGLUT3 polypeptide comprises 20 detecting glutamate transport in a cell expressing a heterologous VGLUT1 polypeptide, VGLUT2 polypeptide or a VGLUT3 polypeptide.

18. The method of claim 1, wherein said test agent is not an antibody.

19. The method of claim 1, wherein said test agent is not a nucleic acid.

20. The method of claim 1, wherein said test agent is not a protein.

25 21. The method of claim 1, wherein said test agent is a small organic molecule.

22. The method of claim 1, wherein said agent is not an agent that alters
 ΔpH or $\Delta\Psi$.

23. The method of claim 1, further comprising comparing the level of
expression or activity of VGLUT1 with the level of expression or activity of VGLUT2 or
5 VGLUT3.

24. A method of prescreening for a potential modulator of glutamate
transporter activity, said method comprising:

contacting a VGLUT glutamate transporter polypeptide or a nucleic
acid encoding a VGLUT glutamate transporter polypeptide with a test agent; and

10 detecting binding of said test agent to said VGLUT glutamate
transporter polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter
polypeptide wherein specific binding of said test agent to the VGLUT glutamate transporter
polypeptide or VGLUT nucleic acid indicates that said test agent is a potential modulator of
glutamate transporter activity.

15 25. The method of claim 24, further comprising recording test agents that
specifically bind to said VGLUT glutamate transporter polypeptide or to said nucleic acid
encoding a VGLUT glutamate transporter polypeptide in a database of candidate
modulators of glutamate transporter activity.

20 26. The method of claim 24, wherein said VGLUT glutamate transporter
polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter is a VGLUT1
polypeptide or a nucleic acid encoding a VGLUT1 polypeptide.

27. The method of claim 24, wherein said VGLUT glutamate transporter
polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter is a VGLUT2
polypeptide or a nucleic acid encoding a VGLUT2 polypeptide.

25 28. The method of claim 24, wherein said VGLUT glutamate transporter
polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter is a VGLUT3
polypeptide or a nucleic acid encoding a VGLUT3 polypeptide.

29. The method of claim 24, wherein said test agent is not an antibody.

30. The method of claim 24, wherein said test agent is not a protein.

31. The method of claim 24, wherein said detecting comprises detecting specific binding of said test agent to said nucleic acid encoding a VGLUT glutamate transporter polypeptide.

5 32. The method of claim 31, wherein said binding is detected using a method selected from the group consisting of a Northern blot, a Southern blot using DNA derived from the VGLUT1, VGLUT2, or VGLUT3 mRNA, an array hybridization, an affinity chromatography, and an *in situ* hybridization.

10 33. The method of claim 24, wherein said detecting comprises detecting specific binding of said test agent to said VGLUT glutamate transporter polypeptide.

34. The method of claim 45, wherein said detecting is via a method selected from the group consisting of capillary electrophoresis, a Western blot, mass spectroscopy, ELISA, immunochromatography, thin layer chromatography, and immunohistochemistry.

15 35. The method of claim 24, wherein said test agent is contacted directly to said VGLUT glutamate transporter polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter polypeptide.

20 36. The method of claim 24, wherein said test agent is contacted to a cell containing said VGLUT glutamate transporter polypeptide or to said nucleic acid encoding a VGLUT glutamate transporter polypeptide.

37. The method of claim 36, wherein said cell is cultured *ex vivo*.

38. A cell comprising a heterologous nucleic acid encoding a glutamate transporter wherein said glutamate transporter is selected from the group consisting of VGLUT1, VGLUT2, and VGLUT3.

25 39. The cell of claim 38, wherein said cell is a mammalian cell.

40. The cell of claim 38, wherein said cell is a somatic cell.

41. The cell of claim 38, wherein said cell is an oocyte or a nerve cell.

42. The cell of claim 38, wherein said cell transports glutamate via said glutamate transporter.

43. The cell of claim 38, wherein said cell is a pheochromocytoma PC12
5 cell.

44. A method of increasing glutamate transport by a mammalian cell, said method comprising transfecting said cell with a nucleic acid encoding a VGLUT polypeptide selected from the group consisting of VGLUT1, VGLUT2, and VGLUT3.

45. The method of claim 44, wherein said nucleic acid encoding a
10 VGLUT polypeptide is operably linked to a constitutive promoter.

46. The method of claim 44, wherein said nucleic acid encoding a
VGLUT polypeptide is operably linked to an inducible promoter.

47. The method of claim 44, wherein said nucleic acid encoding a
VGLUT polypeptide is operably linked to a tissue-specific promoter.

48. A kit for screening for compounds that modulate glutamate transport,
said kit comprising

a cell that expresses a VGLUT glutamate transporter selected from
the group consisting of VGLUT1, VGLUT2, and VGLUT3; and

20 a detection moiety selected from the group consisting of an antibody
that specifically binds to said VGLUT glutamate transporter, a nucleic acid that specifically
binds to a nucleic acid encoding said VGLUT glutamate transporter, a primer that
specifically amplifies a nucleic acid encoding said VGLUT glutamate transporter or a
fragment thereof, and a labeled glutamate.

49. The kit of claim 48, wherein said cell is cell comprising a
25 heterologous nucleic acid encoding said glutamate transporter

50. The kit of claim 48, further comprising instructional materials providing protocols for screening for modulators of a VGLUT glutamate transporter and teaching that such modulators alter glutamate transport.

5 51. A knockout mammal, said mammal comprising a disruption in an endogenous glutamate transporter gene selected from the group consisting of *VGLUT1*, *VGLUT2*, and *VGLUT3*, wherein said disruption results in said knockout mammal exhibiting decreased expression of a VGLUT glutamate transporter as compared to a wild-type animal.

10 52. The mammal of claim 51, wherein the mammal is selected from the group consisting of an equine, a bovine, a rodent, a porcine, a lagomorph, a feline, a canine, a murine, a caprine, an ovine, and a non-human primate.

53. The mammal of claim 51, wherein the disruption is selected from the group consisting of an insertion, a deletion, a frameshift mutation, a substitution, and a stop codon.

15 54. The mammal of claim 51, wherein the disruption comprises an insertion of an expression cassette into said endogenous glutamate transporter gene.

55. The mammal of claim 54, wherein said expression cassette comprises a selectable marker.

20 56. The mammal of claim 54, wherein the expression cassette comprises a neomycin phosphotransferase gene operably linked to at least one regulatory element.

57. The mammal of claim 52, wherein said disruption is in a somatic cell.

58. The mammal of claim 52, wherein said disruption is in a germ cell.

59. The mammal of claim 52, wherein the mammal is homozygous for the disrupted glutamate transporter gene.

25 60. The mammal of claim 52, wherein the mammal is heterozygous for the disrupted glutamate transporter gene.

61. A method of inhibiting glutamate uptake into a cell, said method comprising contacting said cell with an agent that inhibits expression or activity of a VGLUT polypeptide.

62. The method of claim 53, wherein said agent is not an agent that alters
5 ΔpH or $\Delta\Psi$.

63. The method of claim 53, wherein said agent is selected from the group consisting of a VGLUT antisense molecule, a VGLUT ribozyme, a VGLUT catalytic DNA, an anti-VGLUT antibody, and a nucleic acid that disrupts a VGLUT gene by homologous recombination.

10 64. A method of increasing glutamate uptake into a cell, said method comprising contacting said cell with an agent that increases VGLUT glutamate transporter expression or activity.

65. A method of inhibiting glutamate uptake into a cell, said method comprising downregulating expression or activity of a VGLUT polypeptide in said cell.

15 66. The method of claim 65, wherein said inhibiting comprises a method selected from the group consisting of contacting a VGLUT nucleic acid with a ribozyme that specifically cleaves said VGLUT nucleic acid, contacting a VGLUT nucleic acid with a catalytic DNA that specifically cleaves said VGLUT nucleic acid, transfecting a cell comprising an VGLUT gene with a nucleic acid that inactivates the VGLUT gene by
20 homologous recombination with the VGLUT gene, transfecting a cell comprising a nucleic acid encoding an intrabody that specifically binds a VGLUT polypeptide, and transfecting said cell with a VGLUT antisense molecule.